

通光藤甙 F, G, H 和 I 结构*

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摘要 从通光藤 (*Marsdenia tenacissima*) 的茎中分离得到 4 个新的 C_{21} 甙体甙——通光藤甙 F (3), G (4), H (5) 和 I (6), 以及 2 个已知化合物通光藤甙 A (1) 和 B (2)。根据光谱数据和化学方法推定了其结构。同时, 通过 COLOC 谱和二维核磁共振谱指定了这些化合物中甙元上 11 和 12 位的酯基确切连接位置。

关键词 萝藦科, 通光藤, C_{21} 甙体甙, 通光藤甙 F, G, H 和 I

分类号 Q 946

New C_{21} Steroidal Glycosides from *Marsdenia tenacissima*

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Abstract From the stem of *Marsdenia tenacissima* (Roxb) Wight et Arn., four new steroidal glycosides designated tenacissoside F (3), G (4), H (5) and I (6) possessing the same sugar chain: 6 - deoxy - 3 - O - methyl - β - D - allopyranosyl - (1 $\rightarrow$ 4) - β - D - oleandropyranosose, along with two known tenacissoside A (1) and B (2), were isolated and characterized by means of spectroscopic and chemical evidence. Furthermore, diester groups at C - 11 and C - 12 of aglycone of these glycosides had been assigned by COLOC and 2D NMR techniques.

Key words Asclepiadaceae, *Marsdenia Tenacissima*, C_{21} steroidal glycosides, Tenacissosides F, G, H and I

The Yunnan folk medicinal herb "Tong - guang - teng", the stems of *Marsdenia tenacissima* (Roxb) Wight et Arn. (Asclepiadaceae), indigenous to the southwest of China, has been documented to possess functions against inflammation, asthma and cancer, and is widely employed as a therapeutic drug for tonsillitis, pharyngitis, cystitis, pneumonia, asthma and so on (Jiangsu New College of Medicine, 1977; Li, 1995). Shinghal (1980) and co - workers isolated cissogenin and related compounds from *M. tenacissima* native to India. While tenacigenins A, B and C were reported from the acidic hydrolyzate of crude glycoside from *M. tenacissima* collected in China by J. Zhou, and colleagues (Zhou *et al*, 1980; Yang *et al*, 1981). Tenacissosids A, B, C, D and E were illustrated

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from this plant by Miyakawa *et al* (1986). However, the order of diester groups linked at C - 11 and C - 12 of tenacissoside A, C, D and E remained unsettled. In connection with our interest on the biological compound of this plant, four new pregnane glycosides named tenacissoside F (3), G (4), H (5) and I (6) along with tenacissoside A (1) and B (2) were characterized. In the present paper, we wish to describe the isolation and structural elucidation of these glycosides based on spectroscopic and chemical evidence. Furthermore, the COLOC experiment was successfully applied to clarify the exact linkage of diester groups at C - 11 and C - 12 of aglycone for these glycosides in our work.

Tenacissoside A (1) showed a molecular formula $C_{48}H_{74}O_{19}$ from an ion peak at m/z 953 [$(C_{48}H_{74}O_{19}) - H$] $^-$ and combined ^{13}C NMR spectrum (Tables 1 and 2). The mp, ^1H NMR and ^{13}C NMR spectra of 1 were almost the same as those reported (Miyakawa *et al*, 1986). In order to determine the orders of the diester groups, partial alkaline hydrolysis was tried to carry out in various cases, but we failed to obtain partial alkaline hydrolyzate. Fortunately, the assignment of the proton signals at C - 11 and C - 12 were unequivocal. Since the proton at C - 11 forms a triplet due to spin coupling both to the proton at C - 12 and to that at C - 9, whereas the proton at C - 12 forms only a doublet due to spin coupling to the proton at C - 11. Therefore, the COLOC spectrum of 1 was measured to determine the exact linkages of diester groups at C - 11 and C - 12 of 1. In the COLOC spectrum of 1, the proton signal at δ 5.73 (1H, t, $J = 10.1$ Hz, H - 11 β) was correlated with carbon signal at δ 167.37 due to carboxyl carbon of tigloyl group, and the proton signal at δ 5.38 (1H, d, $J = 10.2$ Hz, H - 12 α) was correlated with carbon signal at δ 170.80 due to carboxyl carbon of acetyl group. Thus the tigloyl group of 1 was deduced to link at C - 11 and the acetyl group at C - 12. Consequently, the structure of 1 was established as 11 - α - O - tigloyl - 12 - β - O - acetyltenacigenin B 3 - O - β - D - glucopyranosyl - (1 $\rightarrow$ 4) - 6 - deoxy - 3 - O - methyl - β - D - allopyranosyl - (1 $\rightarrow$ 4) - β - D - oleanodropyranoside.

Tenacissoside F (3) displayed a molecular formula $C_{35}H_{56}O_{12}$ based on elemental analysis. The ^1H NMR spectrum of 3 revealed the signals due to each two secondary methyl and methoxyl groups of sugar moiety at δ 1.54, 1.67, 3.52 and 3.82. The ^1H and ^{13}C NMR spectra of 3 (Tables 1 and 2) due to sugar moiety indicated the presence of two anomeric signals. In addition, all the glycosidic linkages were suggested to be β from the coupling constants of two anomeric proton signals at δ 4.80 (1H, dd, $J = 8.9, 1.5$ Hz) and 5.31 (1H, d, $J = 8.1$ Hz). The ^{13}C NMR spectrum of 3 (Tables 1 and 2) demonstrated that 3 contained pachybiose (10): 6 - deoxy - 3 - O - methyl - (1 $\rightarrow$ 4) - β - D - allopyranosyl - (1 $\rightarrow$ 4) - β - D - oleanodropyranoside (Hayashi *et al*, 1980) as its sugar moiety and tenacigenin B (7) (Miyakawa *et al*, 1986) as its aglycone. This was confirmed by the mild acidic hydrolysis (0.025 mol/L H_2SO_4 - 50 % MeOH, 60 $^\circ$ C, 60 min) of 3 to afford a single sugar component which was identified to be 10 by TLC comparison with authentic sample. On stronger acidic hydrolysis (0.2 mol/L H_2SO_4 - 50% MeOH, 60 $^\circ$ C, 60 min) of 3, it led to oleandrose (8), 6 - deoxy - 3 - O - methylallose (9) and 7 which were determined by TLC comparison with authentic samples. In the negative FABMS of spectrum, besides the quasi - molecular ion peaks at m/z 667 [$M (C_{35}H_{56}O_{12}) - H$] $^-$, typical fragment ion at m/z 503 [M - 6 - deoxy - 3 - O - methylallose] $^-$, 363 [M - pachy-

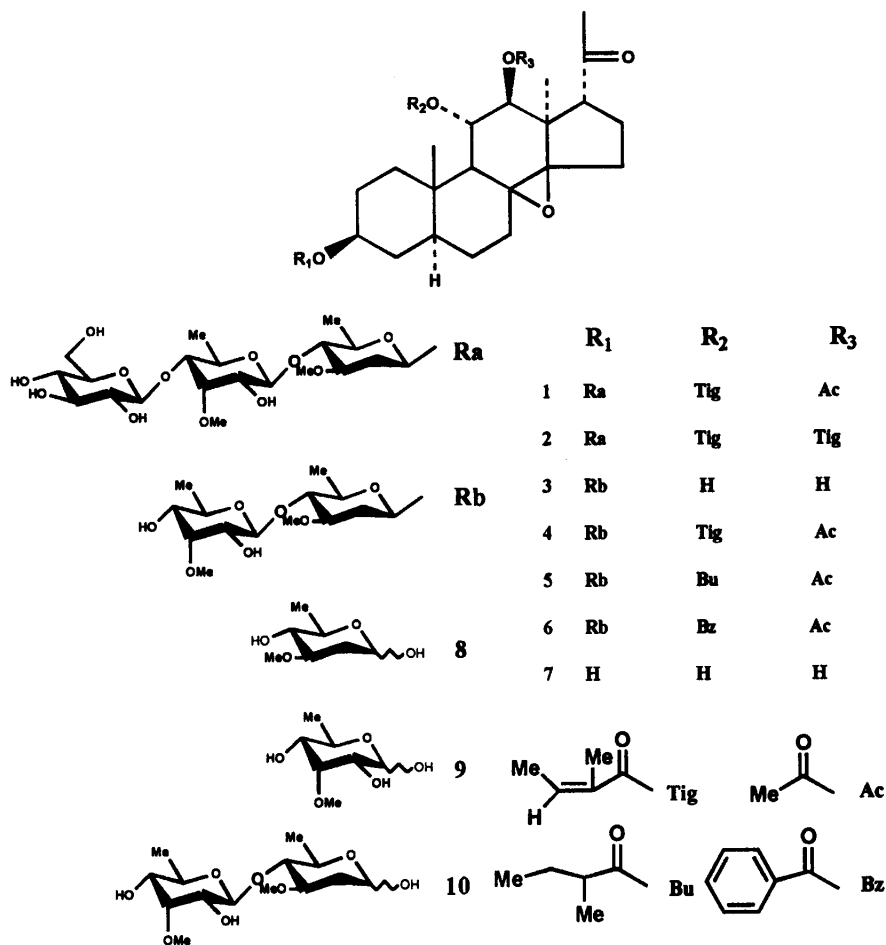
biase - H]⁻ were observed. This indicated that **9** was terminal sugar and **8** was inner sugar. Furthermore, the ¹³C NMR spectral data (Table 2) due to sugar moiety of **3** was in excellent agreement with those of **10** (Hayashi *et al*, 1980). The ¹³C NMR spectra (Table 2) due to sugar moiety of **4**, **5** and **6** coincided exactly with those of **3** so that they were assumed to share the same sugar sequence as that of **3**. The ¹³C NMR spectral data (Table 1) ascribed to the aglycone moiety of **3** corresponded to those of **7** except the aglycone carbons of **3** at C-2 (-2.2 ppm), C-3 (+7.0), and C-4 (-4.1). This glycosidation shifts implied that the sugar chain in **3** was linked to the C-3 hydroxyl group of the aglycone. The same glycosidation shifts were observed in the other glycoside **4**, **5**, and **6**, so that they were shown to bear the sugar moiety at the C-3 hydroxyl group. Thus the structure of **3** was deduced to be tenacigenin B 3-O-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside.

Tenacissoside G (**4**) exhibited a molecular formula C₄₂H₆₄O₁₄ on the terms of its elemental analysis and an ion peak at m/z 791 [M - H]⁻ in its negative FABMS. Two anomeric proton signals at δ 4.50 (1H, dd, J = 8.8, 1.6 Hz) and 4.71 (1H, d, J = 8.2 Hz) and two anomeric carbon signals at δ 97.59 and 101.89 were observed in ¹³C NMR spectrum (Table 2). Mild acidic hydrolysis of **4** afforded **10** which was determined by TLC comparison with authentic sample. Stronger conditions led to **8** and **9**, which were identical with authentic samples by TLC analyses. So **4** possessed the same sugar chain as that of **3** from its ¹H and ¹³C NMR chemical shifts (Table 2). The ¹H NMR spectrum of **4** showed a pattern similar to that of **3** except for the signals of the methine bearing the diester groups (tigloyl and acetyl) which were deduced from the signals at δ 1.67 (3H, d, J = 6.6 Hz, Tig-Me-4), 1.68 (3H, br.s, Tig-Me-5), 6.68 (1H, qq, J = 7.2, 1.9 Hz, Tig-H-3) and 1.77 (3H, s, Ac). The ¹³C NMR chemical shifts (Table 1) also supported the presence of tigloyl and acetyl groups. In the ¹³C-¹H NMR and COLOC spectra of **4**, the proton signal at δ 5.32 (1H, t, J = 10.2 Hz, H-11β) was jointed to the carbon signal at δ 68.61 (C-11) and coupled with the carboxyl carbon signal at δ 167.05 of tigloyl group, and the proton signal at δ 4.92 (1H, d, J = 10.1 Hz, H-12α) was linked to the carbon signal at δ 74.99 (C-12) and correlated with the carboxyl carbon at δ 170.51 of acetyl group. Hence, the structure of **4** was characterized as 11-α-O-tigloyl-12-β-O-acetyltencigenin B 3-O-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside.

Teneaissoide H (**5**) had a molecular formula C₄₂H₆₆O₁₄ on the base of its elemental analysis and an ion peak at m/z 793 (M - H)⁻ in its negative FABMS. On mild acidic hydrolysis, **5** produced one sugar component which was identified as **10** by TLC comparison. On stronger conditions, **5** afforded **8** and **9**, which were identical with authentic samples by TLC analyses. The ¹H NMR spectral data of **5** was similar to those of **3** except for the signals ascribed to 2-methyl butanoyl and acetyl groups at 0.87 (3H, t, J = 7.5 Hz, Bu-Me-4), 0.97 (3H, d, J = 5.5 Hz, Bu-Me-5) (Miyakawa *et al*, 1986) and 1.90 (3H, s, Ac). The ¹³C NMR spectrum (Tables 1 and 2) was also analogous to that of **3** other than the signals due to the diester groups. In the ¹³C-¹H NMR and COLOC spectra of **5**, the proton signal at δ 5.28 (1H, t, J = 10.0 Hz, H-11β) was connected with the carbon signal

at δ 68.44 (C - 11) and spun with the carboxyl group signal at δ 175.36 of 2 - methyl butanoyl group and the proton signal at δ 4.91 (1H, d, J = 10.2 Hz, H - 12 α) was attached to the carbon signal at δ 75.24 (C - 12) and correlated with the carboxyl group signal at δ 170.48 of acetyl group. From these results, we concluded that the structure of **5** was 11 - α - O - 2 - methylbutanoyl - 12 - β - O - acetylenacigenin B 3 - O - 6 - deoxy - 3 - O - methyl - β - D - allopyranosyl - (1 $\rightarrow$ 4) β - D - oleandropyranoside.

Tenacissoside I (**6**) demonstrated a molecular formula $C_{44}H_{62}O_{14}$ based on elemental analysis and an ion peak at m/z 813 [$M (C_{44}H_{62}O_{14}) - H$] $^-$ in its negative FAB - MS. On mild acidic hydrolysis, **6** liberated one sugar fragment identified as **10** by TLC behavior. Stronger acidic hydrolysis of **6** gave **8** and **9** as revealed by TLC comparison with authentic samples. The ^1H NMR spectral data of **6** showed a resemblance to the signal pattern of **3** other than the signals due to benzoyl and acetyl group charac-



terized from the signals at δ 7.36 (2H, t, J = 7.6 Hz, Bz - H - 4,6), 7.50 (1H, t, J = 7.2 Hz, Bz - H - 5), 7.90 (2H, d, J = 8.0 Hz, Bz - H - 3,7) and 2.15 (3H, s, Ac). The ^{13}C NMR spectrum of **6** (Tables 1 and 2) was also similar to that of **3** except for the signals due to benzoyl and acetyl groups. In the ^{13}C - ^1H NMR and COLOC spectra of **6**, the proton signal at δ 5.54 (1H, t, J

δ 10.2 Hz, H-11 β) was joined with the carbon signal at δ 69.58 (C-11) and correlated with the carboxyl carbon signal at δ 165.81 of benzoyl group, and the proton signal δ 5.06 (1H, d, J = 10.2 Hz, H-12 α) was connected with the carbon signal at δ 75.22 (C-12) and correlated with carboxyl carbon signal at δ 170.52 due to acetyl group. Thus, structure of **6** was elucidated as 11- α -O-benzoyl-12- β -O-acetyl tenacigenin B 3-O-6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranoside.

EXPERIMENTAL

Melting points were determined on a PHMK 79/2288 micro-melting point apparatus and uncorrected. Optical rotations were measured with a SEPA-300 polarimeter at room temp. UV spectra were obtained in EtOH with a Shimadzu UV-210 spectrometer and absorption maxima are given in nm. IR spectra were recorded in KBr on a Perkin-Elmer 577 spectrometer. FABMS were run on a VG AUTOSPEC 3000 mass spectrometer. The NMR spectra were run on Bruker AM-400 instrument at 400 MHz for ^1H NMR and 100.6 MHz for ^{13}C NMR in $\text{C}_5\text{D}_5\text{N}$ and chemical shifts were given as δ (ppm) with TMS as an internal standard. Column chromatography were carried out on Qingdao silica gel (200-300 mesh), MCI CHP-20 gel, FUJI (ODS-Q₃) gel (Mitsubishi Chemical Co.) and Lobar Rp-18 gel (Merck). TLC were performed on Merck precoated kiesselgel 60 F₂₅₄ plate, and Rp-18 F₂₅₄ plate with the following solvent system: A. MeOH-CHCl₃ (5:95 and 10:90, v/v), B. Me₂CO-petrol (2:3), and C. H₂O-MeOH (2:8 and 3:7).

Plant material. The plant used in research was collected in January 1993 in Xishuangbanna, Yunnan, China and was identified as *Marsdenia tenacissima* (Roxb) Wight et Arn. by Mr. Jingyun Cui of Xishuangbanna Tropical Botanic Garden, the Chinese Academy of Sciences. The voucher specimen is deposited in the herbarium of this Garden.

Extraction and Isolation of Glycosides. Ground stem of *M. tenacissima* were air-dried, pulverized (2.0 kg), and extracted with 70% EtOH and heated under reflux. A brown tar given by concentration of the extract was dissolved in MeOH to afford MeOH-soluble fraction which was evaporated in vacuo to produce a crude yellow glycoside mixture (126 g). The crude glycosides was subjected to CC on silica gel with solvents of increasing polarity from CHCl₃ to 15% MeOH-CHCl₃ into fraction I (15 g), II (8 g), III (20 g), IV (18 g), V (45 g) and VI (12 g). 1.0 g of fraction III was chromatographed on Lichroprep RP-18 column with MeOH-H₂O (8:2) to separate fraction III-1 (180mg, mainly **4**) and fraction III-2 (600 mg, a mixture of **5** and **6**). The fraction III-1 was rechromatographed on FUJI gel column with MeOH-H₂O (3:1) to afford chromatographically pure **4** (136 mg). The fraction III-2 was submitted to a medium pressure silica gel with Me₂CO-petrol (2:8) to give pure **5** (180mg) and **6** (220 mg). The fraction IV (18 g) was chromatographed on MCI CHP-20 gel with MeOH-H₂O (8:2) to separate fraction IV-1 (2.3 g) and fraction IV-2 (4.0 g). 0.5 g of the fraction IV-1 was further purified by Lichroprep RP-18 column with solvents of MeOH-H₂O (7:3) to give **3** (240 mg). 5.0 g of the fraction V (45.2 g) was chromatographed on

MCI gel and Lichroprep RP-18 (solvent H₂O-MeOH, 7:3) to produce fraction V-1 (mainly 1), V-2, V-3 (mainly 2), V-4. The fractions V-1 and V-3 were successively chromatographed on FUJI gel column with MeOH-H₂O (7:3) and on silica gel column with MeOH-CHCl₃ (5:95) to give pure 1 (300 mg), 2 (150 mg), respectively.

Tenacissoside A (1). An amorphous white powder; mp 139.5~140.5°C; FABMS (negative) m/z: 953 [M(C₄₈H₇₄O₁₉)-H]⁻, 871 [M-83]⁻, 793 [M-161]⁻, 611 [M-343]⁻; ¹H NMR (C₅D₅N): δ 1.20 (3H, s, H-19), 1.23 (3H, s, H-18), 1.58, 1.60, (each 3H, d, J = 6 Hz, H-6 of sugar moiety), 1.73 (3H, br. s, H-4'). 1.76 (3H, s, H-5'), 1.90 (3H, s, H-2''), 2.25 (3H, s, H-21), 2.92 (1 H, br. d, J = 6.2 Hz, H-17β), 3.48, 3.78 (each 3H, s, 3-O-Me of sugar moiety), 4.75 (1H, dd, J = 9.3, 1.8 Hz, Ole-H-1), 4.89 (1H, d, J = 7.6 Hz, Glc-H-1), 5.21 (1H, d, J = 7.9 Hz, Allo-H-1), 5.38 (1H, d, J = 10.2 Hz, H-12α), 5.73 (1H, t, J = 10.1 Hz, H-11β) 6.82 (1H, qq, J = 7.1, 1.8 Hz, H-3'); ¹³C NMR: see Tables 1 and 2.

Table 1 ¹³C NMR Chemical Shifts of the Aglycone Moiety of Glycoside 1-6 and 7

C	1	2	3	4	5	6	7
1	37.87	37.80	37.67	37.72	37.76	37.703	8.3
2	29.93(-2.1)	29.93(-2.1)	29.95(-2.0)	29.85(-2.2)	29.62(-2.4)	29.98(-2.0)	32.0
3	76.32(+6.5)	76.33(+6.5)	76.81(+7.0)	76.18(+6.2)	75.95(+6.2)	76.06(+6.3)	69.8
4	35.40(-4.0)	35.39(-4.0)	35.33(-4.1)	35.29(-4.1)	35.26(-4.1)	35.30(-4.1)	39.4
5	44.14	44.12	44.94	44.03	44.04	44.03	44.3
6	27.31	27.34	28.41	27.24	27.07	27.19	27.0
7	25.39	25.38	25.62	25.30	25.16	25.27	25.1
8	66.93	66.99	66.21	66.81	66.78	66.87	66.8
9	52.01	52.00	54.64	51.89	51.81	51.95	51.8
10	39.48	39.49	39.20	39.41	39.46	39.49	39.4
11	69.26	69.36	68.15	69.12	68.95	69.82	68.9
12	75.27	75.18	74.55	75.19	75.24	75.22	75.2
13	46.32	46.55	48.27	46.21	46.25	46.28	46.2
14	71.88	71.95	71.98	71.75	71.64	71.02	71.6
15	32.29	32.28	32.94	32.22	32.12	32.24	32.1
16	27.21	27.34	27.74	27.24	27.25	27.19	27.2
17	59.93	60.05	60.83	59.81	60.17	59.86	60.0
18	13.19	13.19	13.31	13.31	13.09	13.13	13.2
19	17.04	17.06	18.38	16.97	16.99	16.89	17.0
20	210.47	210.60	211.27	210.28	209.89	210.61	209.8
21	30.42	30.46	32.94	30.33	29.99	30.19	29.8
	Tig	Tig		Tig	Bu	Bz	
1'	167.35	167.64		167.24	175.51	166.18	
2'	129.25	129.30		129.15	41.53	130.93	
3'	138.26	138.34		138.16	26.57	129.92	
4'	12.22	12.08		12.19	11.80	129.06	
5'	14.43	14.34		14.40	15.64	133.55	

续表 1

C	1	2	3	4	5	6	7
6'						129.06	
7'						129.92	
	Ac	Tig		Ac	Ac	Ac	
1"	170.80	167.64		170.68	170.70	170.52	
2"	20.61	128.58		20.55	20.81	20.91	
3"		138.15					
4"		12.08					
5"		14.34					

Glycosidation shifts given in the parentheses by being compared with reported values of 7; Assignments on the basis of $^1\text{H} - ^1\text{H}$, $^{13}\text{C} - ^1\text{H}$ Correlated 2D spectroscopy (COSY) and Distortionless Enhancement by Polarization Transfer (DEPT); Tig: Tigloyl; Bz: Benzoyl; Bu: 2-methylbutanoyl; Ac: acetyl.

Table 2 ^{13}C NMR chemical shifts for the sugar moiety of glycoside 1 ~ 6

C	1	2	3	4	5	6
Ole C - 1	97.73	97.73	97.73	97.59	97.53	97.50
2	37.87	37.86	39.15	37.75	38.15	37.92
3	79.77	79.28	79.67	79.64	79.66	79.62
4	83.16	83.19	83.13	83.02	83.05	83.73
5	71.88	71.95	71.98	71.98	72.04	71.98
6	18.38	18.38	18.64	18.68	18.61	18.61
OMe - 3	57.24	57.23	57.07	57.07	57.02	57.27
Allo C - 1	101.81	101.85	101.90	101.89	101.84	101.82
2	72.01	72.04	73.31	73.24	73.24	73.28
3	83.16	83.19	83.99	83.94	83.92	83.93
4	83.45	83.19	74.61	74.57	74.59	74.60
5	69.61	69.63	71.03	71.01	71.04	71.02
6	19.18	19.17	18.14	19.15	19.15	19.08
OMe - 3	61.80	61.80	62.05	62.11	62.03	62.03
Glc C - 1	106.48	106.51				
2	75.48	75.52				
3	78.34	78.39				
4	71.88	72.04				
5	78.25	78.38				
6	63.13	63.14				

Assignments on the basis of $^1\text{H} - ^1\text{H}$, $^{13}\text{C} - ^1\text{H}$ COSY and DEPT. Ole: β -D-oleandropyranosyl; Allo: 6-deoxy-3-O-methyl- β -D-allopyranosyl; Glc: β -D-glucopyranosyl.

Tenacissoside B (2). An amorphous powder; mp 132 - 134°C; FAB - MS (negative) m/z : 997 $[\text{M}(\text{C}_{51}\text{H}_{82}\text{O}_{19}) - \text{H}]^-$, 915 $[\text{M} - 83]^-$, 837 $[\text{M} - 161]^-$, 665 $[\text{M} - 343]^-$; ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$): see Tables 1 and 2.

Tenacissoside F (3). An amorphous white powder; mp 115 ~ 118°C; $[\alpha]_D^{27} - 28.33^\circ$ (c 0.59, MeOH); Found: C, 62.63; H, 8.54. $\text{C}_{35}\text{H}_{56}\text{O}_{12}$. H_2O requires: C, 62.87; H, 8.54%; IR ν_{max}

cm^{-1} 3450 (OH), 2960, 2930, 1690 (C=O), 1440, 1360, 1300, 1155, 1120, 1100~1040 (C-O-C); FABMS (negative) m/z : 667 [$M(\text{C}_{35}\text{H}_{56}\text{O}_{12}) - \text{H}$] $^{-}$, 507 ($M - 161$) $^{-}$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$): δ 1.16 (3H, s, H-19), 1.44 (3H, s, H-18), 1.54, 1.67 (each 3H, d, $J = 6$ Hz, H-6 of sugar moiety), 2.34 (3 H, s, H-21), 3.48 (1H, br. d, $J = 10.1$ Hz, H-17 β), 3.52, 3.82 (each 3H, s, OMe-3 of sugar moiety), 3.70 (1H, d, $J = 9.4$ Hz, H-12 α), 4.06 (1H, t, $J = 3.0$ Hz, Allo-H-3), 4.41 (1H, t, $J = 10.1$ Hz, H-11 β), 4.80 (1H, dd, $J = 8.9, 1.5$ Hz, Ole-H-1), 5.31 (1H, d, $J = 8.1$ Hz, Allo-H-1); ^{13}C NMR: see Tables 1 and 2.

Tenacissoside G (4). An amorphous white powder; mp 117~119 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{26} + 17.93^{\circ}$; (c 0.46, MeOH); Found: C, 62.78; H, 8.09. $\text{C}_{42}\text{H}_{64}\text{O}_{14} \cdot 1/2 \text{H}_2\text{O}$ requires: C, 62.92; H, 8.11%; FABMS (negative) m/z : 791 [$M(\text{C}_{42}\text{H}_{64}\text{O}_{14}) - \text{H}$] $^{-}$, 749 [$M - 43$] $^{-}$, 709 [$M - 83$] $^{-}$, 631 [$M - 161$] $^{-}$, 667 [709-43] $^{-}$; UV λ_{max} nm (log ϵ): 219.5 (3.96); IR ν_{max} cm^{-1} : 3600-3400 (OH), 2960, 2920, 1725 (C=O), 1700 (C=O), 1640 (C=C), 1440, 1360, 1255, 1155, 1120, 1100-1040 (C-O-C); ^1H NMR (CDCl_3): δ 0.97 (3H, s, H-19), 1.02 (3H, s, H-18), 1.17, 1.28 (each 3H, d, $J = 6$ Hz, H-6 of sugar moiety), 1.67 (3H, d, $J = 6.6$ Hz, H-4'), 1.68 (3H, s, H-5'), 1.77 (3H, s, H-2''), 2.10 (3H, s, H-21), 2.85 (1H, br. d, $J = 7.3$ Hz, H-17 β), 3.11 (1H, m, H-3 α), 3.29, 3.60 (each 3H, s, OMe-3 of sugar moiety), 3.70 (1H, t, $J = 3.0$ Hz, Allo-H-3), 4.50 (1H, dd, $J = 8.8, 1.6$ Hz, Ole-H-1), 4.71 (1H, d, $J = 8.2$ Hz, Allo-H-1), 4.92 (1H, d, $J = 10.1$ Hz, H-12 α), 5.32 (1H, t, $J = 10.2$ Hz, H-11 β), 6.68 (1H, qq, $J = 7.2, 1.9$ Hz, H-3'); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$): see Tables 1 and 2.

Tenacissoside H (5). An amorphous white powder; mp 178~182 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{26} - 14.51^{\circ}$ (c 0.45, MeOH); Found: C, 61.90; H, 8.13. $\text{C}_{42}\text{H}_{66}\text{O}_{14} \cdot \text{H}_2\text{O}$ requires: C, 62.07; H, 8.37%; FABMS (negative) m/z : 793 [$M(\text{C}_{42}\text{H}_{66}\text{O}_{14}) - \text{H}$] $^{-}$, 751 [$M - 43$] $^{-}$, 709 [$M - 85$] $^{-}$, 633 [$M - 161$] $^{-}$; IR ν_{max} cm^{-1} : 3600-3400 (OH), 2960, 2930, 2870, 1725 (C=O), 1695 (C=O), 1242, 1100-1050 (C-O-C); ^1H NMR (CDCl_3): δ 0.81 (3H, t, $J = 7.5$ Hz, H-4'), 0.97 (3H, d, $J = 5.5$ Hz, H-5'), 0.98 (3H, s, H-19), 1.00 (3H, s, H-18), 1.24, 1.30 (each 3H, $J = 6$ Hz, H-6 of sugar moiety), 1.90 (3H, s, H-2''), 2.13 (3H, s, H-21), 2.86 (1H, br. d, $J = 7.4$ Hz, H-17 β), 3.33, 3.59 (each 3H, s, OMe-3 of sugar moiety), 3.72 (1H, t, $J = 3.0$ Hz, Allo-H-3), 4.52 (1H, dd, $J = 9.7, 1.8$ Hz, Ole-H-1), 4.72 (1H, d, $J = 8.3$ Hz, Allo-H-1), 4.91 (1H, d, $J = 10.2$ Hz, H-12 α), 5.28 (1H, t, $J = 10.0$ Hz, H-11 β); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$): see Tables 1 and 2.

Tenacissoside I (6). An amorphous powder; mp 178~182 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{26} - 18.42^{\circ}$, (c 0.45, MeOH); Found: C, 63.22; H, 7.53. $\text{C}_{44}\text{H}_{62}\text{O}_{14} \cdot \text{H}_2\text{O}$ requires: C, 63.46, H, 7.69. FABMS (negative) m/z : 813 [$M(\text{C}_{44}\text{H}_{62}\text{O}_{14}) - \text{H}$] $^{-}$, 653 [$M - 161$] $^{-}$; UV λ_{max} nm (log ϵ): 230.5 (4.03); IR ν_{max} cm^{-1} : 3460 (OH), 2960, 2930, 1730 (C=O), 1700 (C=O), 1590, 1575, 1440, 1360, 1310, 1270, 1235, 1100-1050 (C-O-C), 830, 760, 710; ^1H NMR (CDCl_3): δ

1.06 (3H, s, H-19), 1.09 (3H, s, H-18), 1.20, 1.27 (each 3H, $J = 6.0$ Hz, H-6 of sugar moiety), 1.54 (3H, s, H-2''), 2.15 (3H, s, H-21), 2.90 (1H, br. d, $J = 7.3$ Hz, H-17 β), 3.31, 3.60, (each 3H, s, OMe-3 of sugar moiety), 3.73 (1H, t, $J = 3.0$ Hz, Allo-H-3), 4.50 (1H, dd, $J = 9.7, 1.8$ Hz, Ole-H-1), 4.73 (1H, d, $J = 8.2$ Hz, Allo-H-1), 5.06 (1H, d, $J = 10.2$ Hz, H-12 α), 5.54 (1H, t, $J = 10.2$ Hz, H-11 β), 7.36 (2H, t, $J = 7.6$ Hz, H-4', 6'), 7.50 (1H, $J = 7.2$ Hz, H-5'), 7.90 (2H, d, $J = 8.0$ Hz, H-3', 7'); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$): see Tables 1 and 2.

Mild acidic hydrolysis of **1** ~ **6** with 0.025 mol/L H_2SO_4 - 50% MeOH. A soln of **3** (5 mg) in MeOH (3 mL) and 0.1 mol/L H_2SO_4 (1 mL) was kept at 60°C for 30 min, then H_2O (3 mL) was added and the whole was concd to 4 mL. The soln was warmed at around 60°C for further 30 min and neutralized with $\text{Ba}(\text{OH})_2$ aq. soln. The ppt was filtered off and the filtrate was evaporated to dryness to give a mixture of hydrolyzate of **3**. The other three glycosides **4** ~ **6** were acid-hydrolysed by the procedure described above. Every hydrolyzate contained one sugar fragment identified as pachybiose (**10**) by TLC comparison with authentic samples.

Stronger acidic hydrolysis of **3** ~ **6** with 0.1 mol/L H_2SO_4 . To a soln of 5 mg of **3** in 2 mL MeOH was added 2 mL of 0.2 mol/L H_2SO_4 and the mixture kept at 60°C for 1 hr. After work-up, tenacigenin B (**7**), oleandrose (**8**), 6-deoxy-3-O-methyl-allose (**9**) were detected by TLC with authentic samples. The other 3 glycosides (**4** ~ **6**) were acid-hydrolyzed by the same procedure described above. Every hydrolyzate demonstrated **8**, **9** and its aglycone by TLC comparison with authentic samples.

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